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Measurement uncertainty in quantitative metabolomics

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Outline

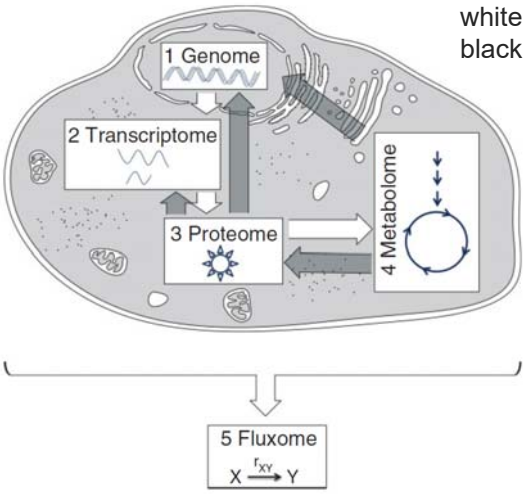
- Biological and industrial relevance
- Metabolomics and measurements
- Concentrations and fluxes
- Alternative metabolic pathways
- Analytical approach
- Uncertainty of measurement and correction

Biochemical Studies of Living Cells

- Bacteria, fungi, mammalian, plant
- Understanding metabolism
- Understanding the interactions between compartments in cells
- Understanding the effect of extraneous chemicals (drugs, poisons) on the cell
- Producing chemicals, proteins and drugs from cell cultures
- Enhancing these production pathways

Cell compartments and interactions

white arrows …. flow of information
black arrows …. regulations



S. Klein and E. Heinzle, 2012

Metabolomics

- Provides absolute or relative metabolite levels (intracellular or extracellular)
- BUT: Concentration changes cannot unambiguously be interpreted as changes of metabolic rates (fluxes)
- Increase in concentration can be from:
  - Increased activity of producing enzymes
  - Increased pool (reservoir) within cell
  - Decreased activity of consuming enzymes

J. M. Buescher et al., 2015

The measurement problem

- Small quantities of cell mass (mg)
- Numerous small molecules involved in metabolic pathway
- Most of the molecules are highly reactive
- Temporary development of concentration profiles is relevant
- Difficult to „freeze" the temporary state
- Temporary state inferred from degree of labelling of molecules via ^{13}C glucose

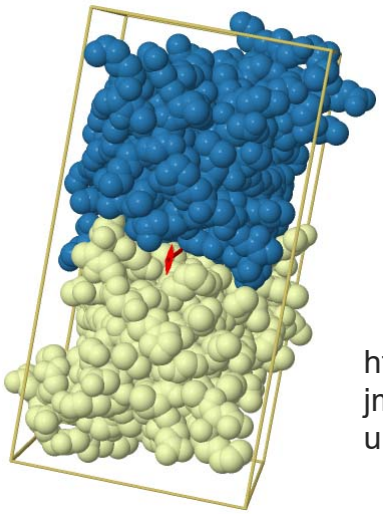
Analytical solution

42 analytes in *Pichia pastoris* broth for production of human superoxide dismutase

- Quenching of metabolism: sampling of cell broth into 60% MeOH at -30° C
- Filtered and stored at -80° C
- Extracted with 4 ml EtOH (75%) for 3 min at 85° C
- Automated ethoximation and trimethylsilylation
- GC-CI-TOFMS

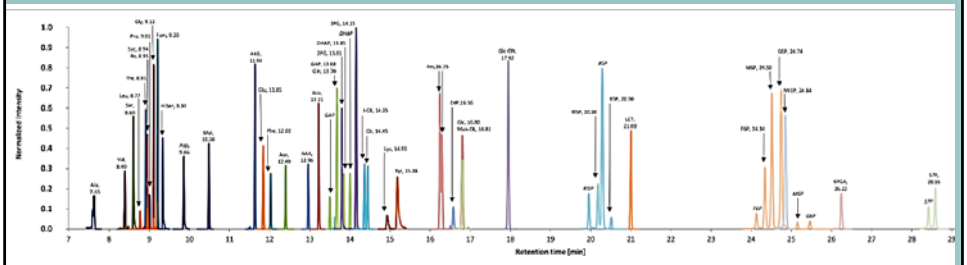
T. Mairinger et al., 2015

Human Cu-Zn superoxide dismutase mutant G93A



http://www.rcsb.org/pdb/explore/jmol.do?structureId=2ZKY&biounumber=5&view=symmetry

GC-run

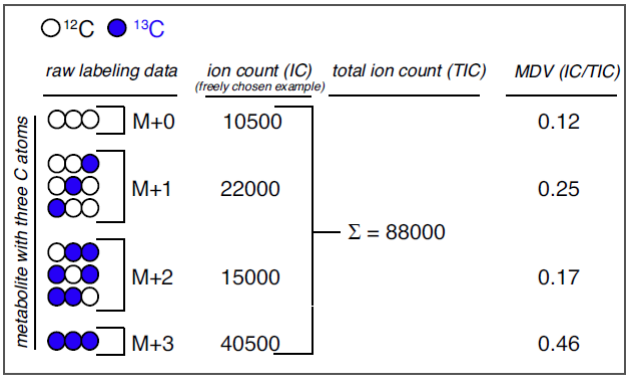


- Amino acids
- Sugars
- Phosphates
- Intermediates

T. Mairinger et al., 2015

Mass spectrometric pattern from isotopologues

- 13-C labelling for a C-3 molecule
  - Random statistical frequency 1:3:3:1

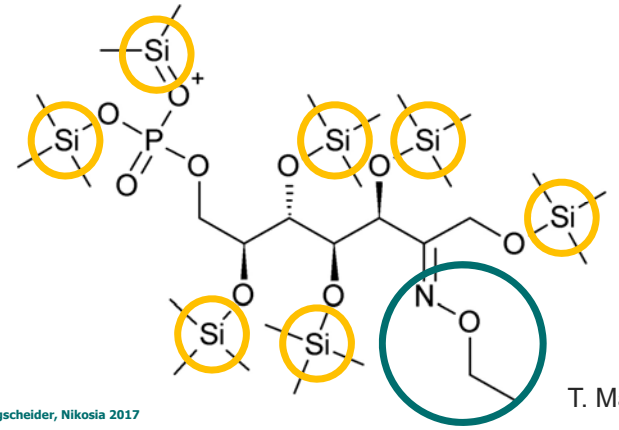


- The bigger the molecule the more isotopologues

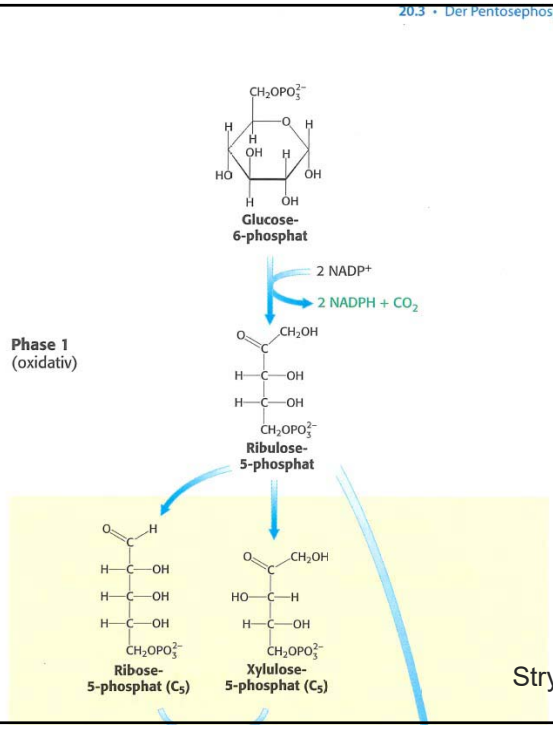
J.M. Buescher et al., 2015

One of 42 analytes:
Sedoheptulose-7-phosphate

- 7 Trimethylsilyl and 1 Ethoximate
- 29 C atoms and 7 Si atoms



T. Mairinger et al., 2015

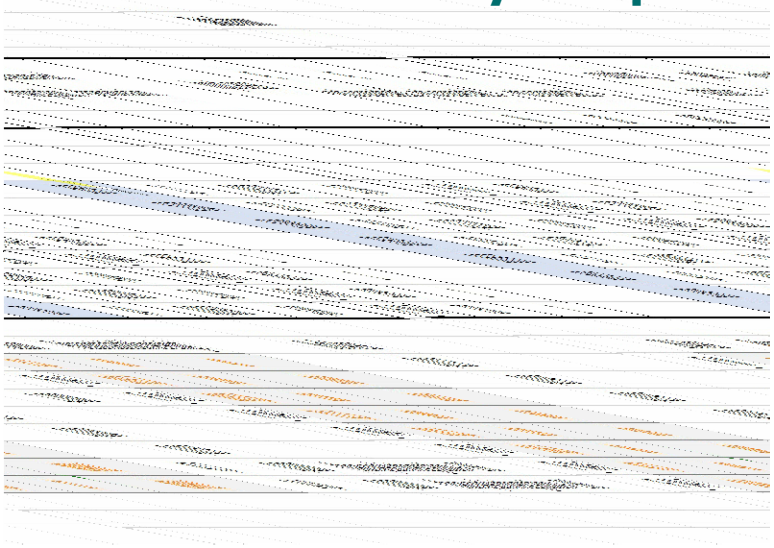


Stryer Biochemie, 2013

Components of uncertainty

- From analytical protocol:
  - Ionization and transmission efficiency
  - Poisson statistics
  - Background subtraction
  - Integration
- From naturally occurring isotopes:
  - ^{13}C in backbone and derivatization reagent
  - ^{28}Si, ^{29}Si, ^{30}Si in derivatization reagent

Excel solution for analytical protocol

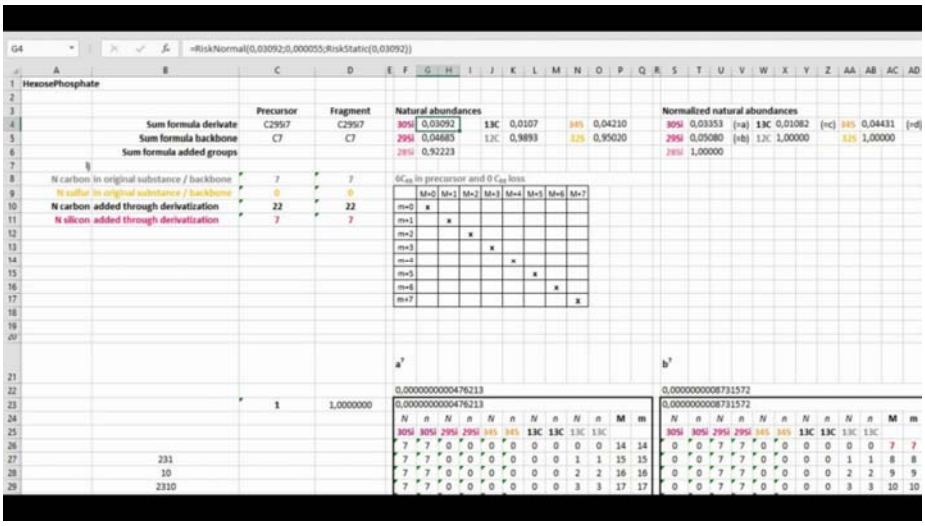


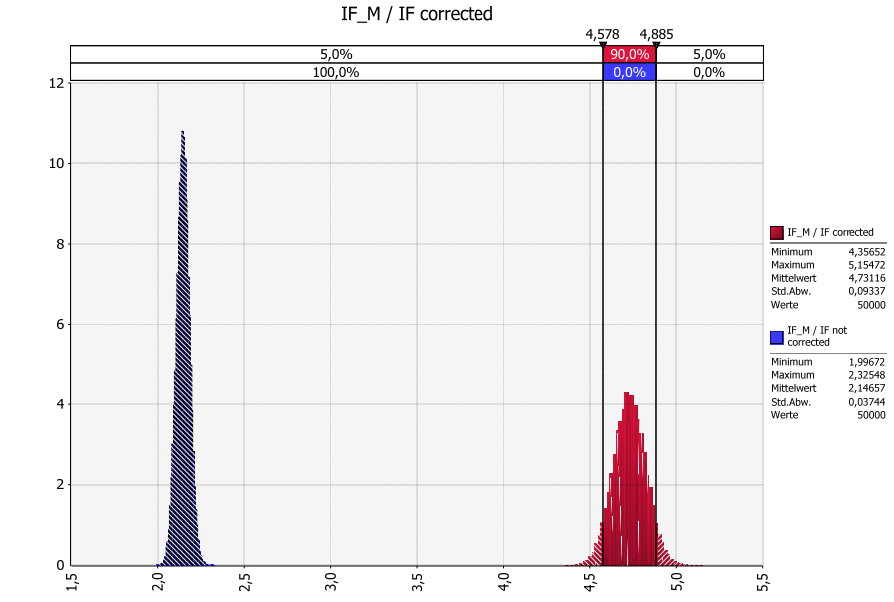
Excel solution for naturally occurring isotopes

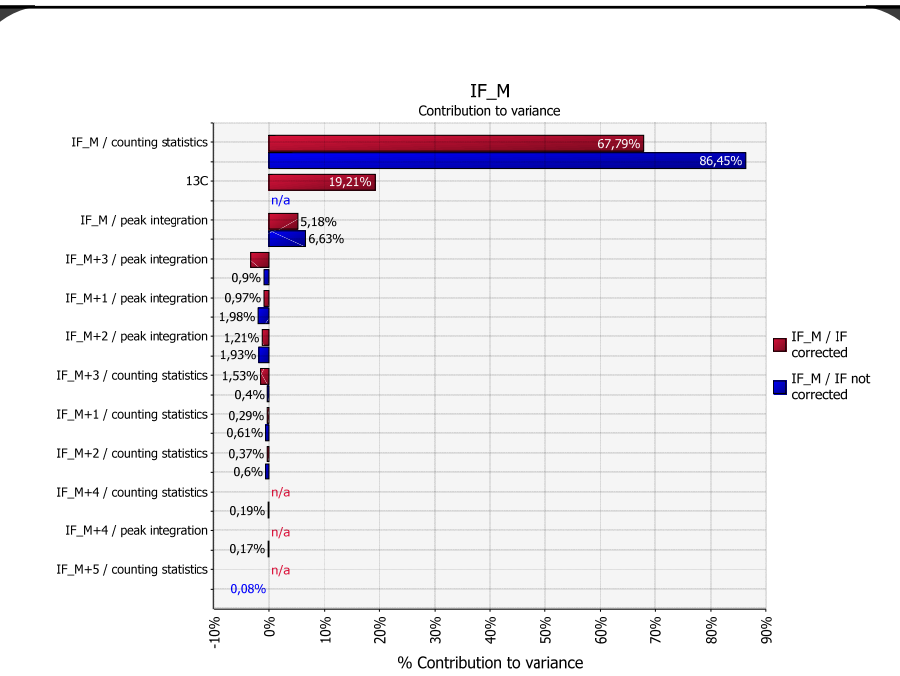
Data from:

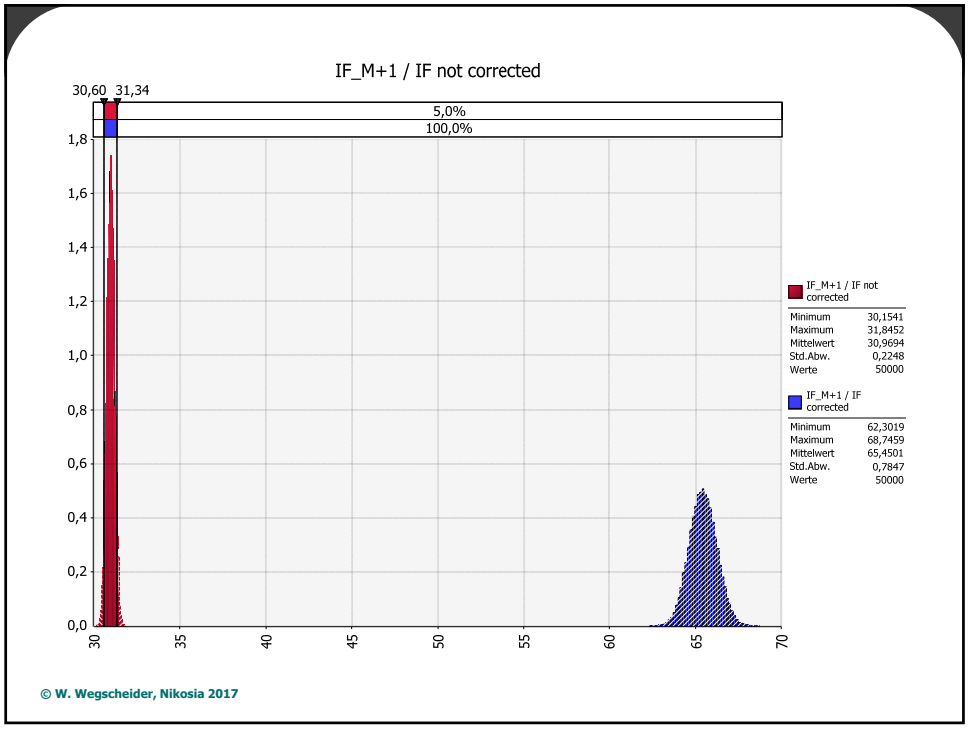
	counting statistics	peak integration	ionization and transmission	Input areas	IF not corrected %	IF not corrected SD	IF not corrected RSD
IF_M	3659	1	1	3659	2,1	0,03729	1,7%
IF_M+1	52784	1		52784	31,0	0,2246	0,7%
IF_M+2	51881	1		51881	30,4	0,2228	0,7%
IF_M+3	35625	1		35625	20,9	0,1797	0,9%
IF_M+4	16555	1		16555	9,7	0,10417	1,1%
IF_M+5	6813	1		6813	4,0	0,05589	1,4%
IF_M+6	2385	1		2385	1,4	0,03109	2,2%
IF_M+7	738	1		738	0,4	0,01638	3,8%

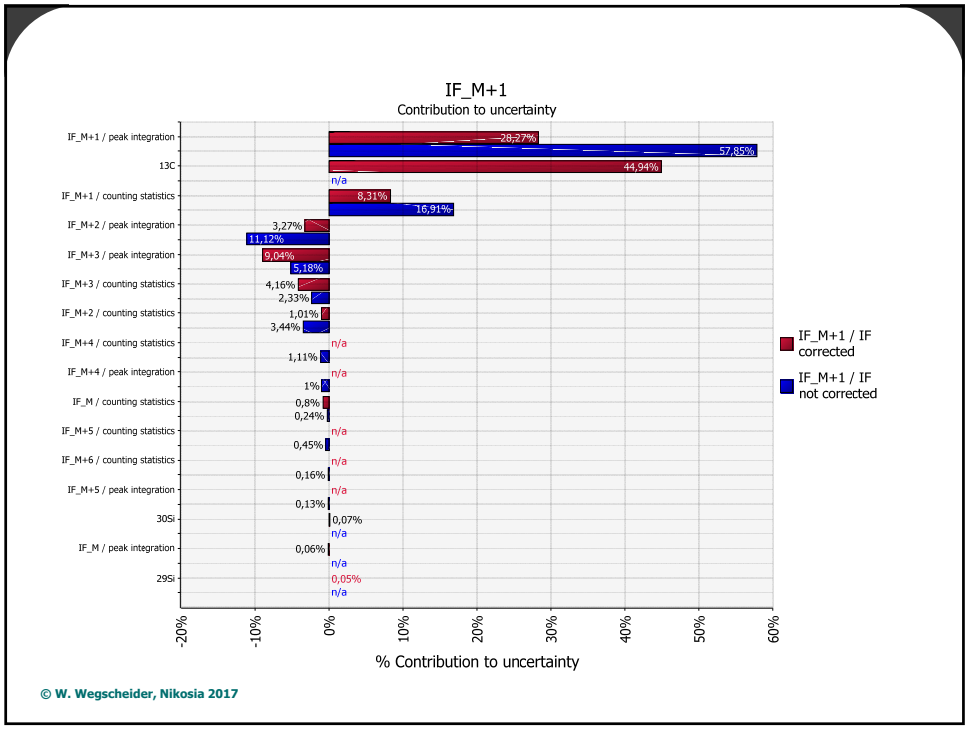
Monte Carlo Simulation

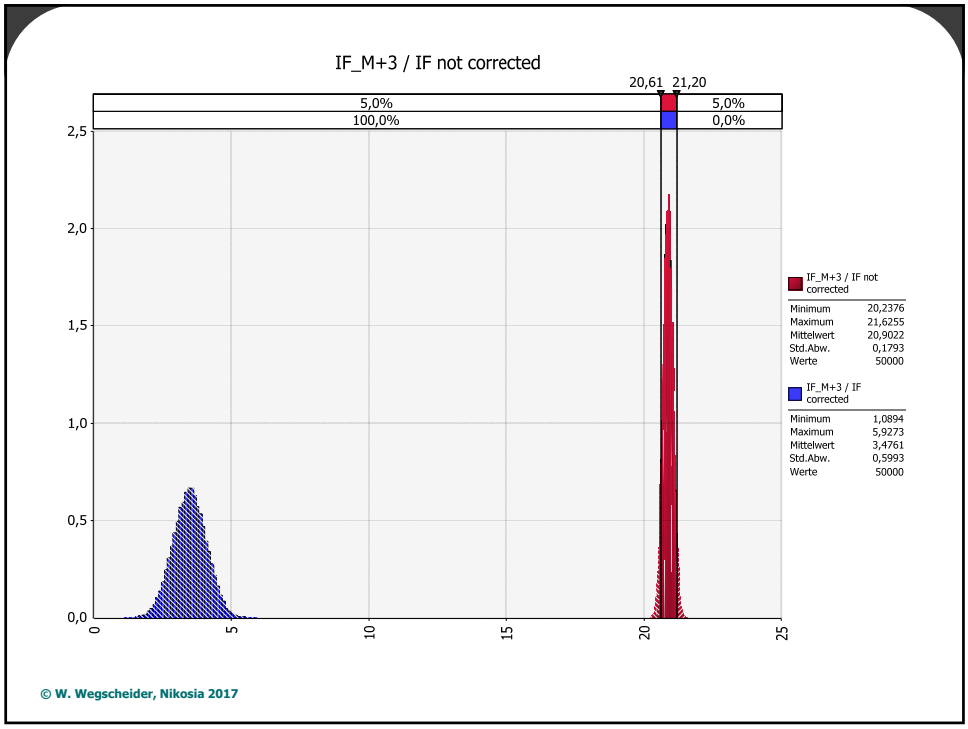


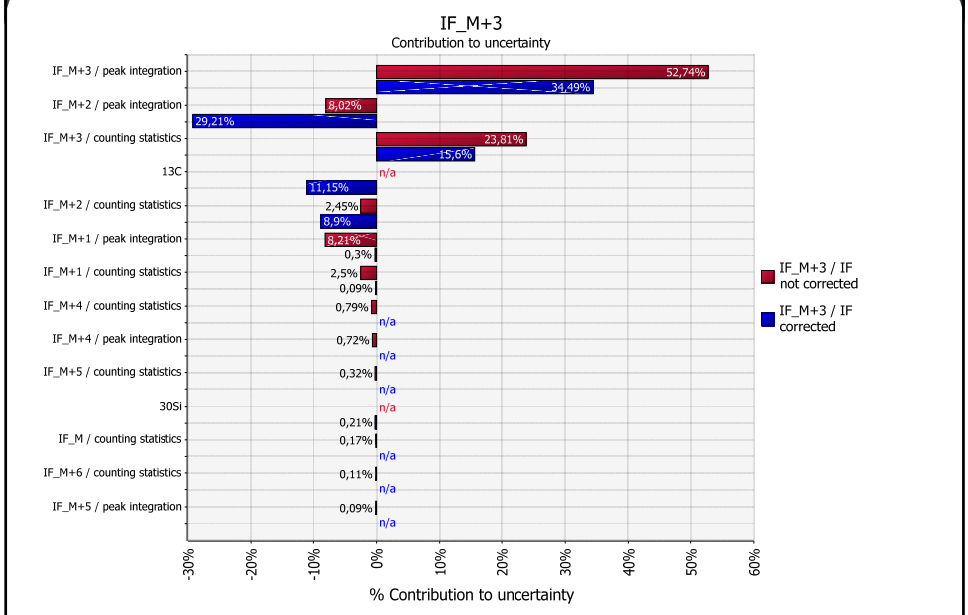












Results for Sedoheptulose Phosphate

mass	IF uncorr	u(IFuncorr)	IF corr	u(IF corr)
IF_M	2,1	0,04	4,7	0,09
IF_M+1	31,0	0,23	65,4	0,78
IF_M+2	30,4	0,22	26,3	0,79
IF_M+3	20,9	0,18	3,5	0,60
IF_M+4	9,7	0,10	-1,7	0,35
IF_M+5	4,0	0,06	-0,7	0,22
IF_M+6	1,4	0,03	-0,2	0,10
IF_M+7	0,4	0,02	0,0	0,05

Conclusions

- The analytical protocol can be considered fairly robust
- The corrections lead to significantly different „true" isotopic signature from labelling
- The corrections for natural isotopic abundances cause an increase of uncertainty of about a factor of 3 - 4
- The significance of these corrections on the flux modelling is not yet established